REMARKS

Applicants are amending their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have incorporated the subject matter of claim 9 into claim 1; and, correspondingly, Applicants have cancelled claim 9 without prejudice or disclaimer.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the references applied by the Examiner in the Office Action mailed December 15, 2008, in rejecting claims as formerly in the application, that is, the teachings of Ovyn, et al., U.S. Patent No. 6,110,681, and the articles by Whitcombe, et al., "A homogenous fluorescence assay for PCR amplicons: Its application to real-time, single-tube genotyping", in Clinical Chemistry, 44:5 (1998), pages 918-923; Rizzo, et al., "Chimeric RNA-DNA molecular beacon assay for ribonuclease H activity", in Molecular and Cellular Probes (2002) 16, 277-283; Leone, et al., "Molecular beacon probes combined with amplification by NASBA enable homogenous, real-time detection of RNA", in Nucleic Acids Research, 1998, Vol. 26, No. 9, pages 2150-2155 (Leone '98); Leone, et al., "Direct detection of potato leafroll virus in potato tubers by immunocapture and the isothermal nucleic acid amplification method NASBA", in Journal of Virological Methods 66 (1997), pages 19-27 (Leone '97); and Uematsu, et al., "Multiplex polymerase chain reaction (PCR) with color-tagged module-shuffling primers for comparing gene expression levels in various cells", in Nucleic Acids Research, 2001, Vol. 29, No. 16, pages 1-6 (August 15, 2001) (as characterized by the Examiner, "Uematsu 2001"), under the provisions of 35 USC 103.

It is respectfully submitted that the references as applied by the Examiner would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, having processing steps as in claim 1, including, inter alia, subjecting a gene to be analyzed to nucleic acid amplification using, inter alia, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene being further defined, and a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease, with two or more target genes being simultaneously detected in a single reaction vessel using two or more types of probes, the two or more target genes being derived from different samples, and wherein each of the two or more types of probes comprise several module sequences of three or four bases, both of the terminal bases of each module sequence being identical to each other and each probe being constituted by rearranging the order of the module sequences having identical terminal bases, and the melting temperatures (Tm values) of the two or more types of probes are substantially the same. See claim 1.

As discussed further infra , the present invention has advantages over the applied prior art, including Whitcombe, et al., in, inter alia, avoiding the need for switching temperatures before cycle 4, as seen in Figure 1 of Whitcombe, et al.

Moreover, as also discussed further infra, according to the present invention each of the two or more probes used in the present invention include several module sequences of three or four bases, both of the terminal bases of each module sequence being identical to each other, and each probe being constituted by

rearranging the order of the module sequences having identical terminal bases. By this specific structure of the probes, while the entire sequence of each probe is different from each other, these probes have substantially the same Tm (melting temperature) value, and can hybridize to their complementary sequences with the same reaction properties, and therefore they can be allowed to simultaneously react in a same reaction tube. Moreover, accurate quantitative analysis of two or more different target genes, even from different samples, is achieved by the present invention. Accordingly, accurate analysis can be made when the probes recited in the present claims are used for quantitative analysis.

In addition, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and, moreover, wherein the two or more types of probes respectively have fluorophores at one end that emit light at fluorescent wavelengths different from each other (see claim 15); and/or wherein a number of module sequences constituting each probe is in a range of 5-8 (see claim 16).

Moreover, it is respectfully submitted that the teachings of the references applied by the Examiner would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having features as in the remaining dependent claims being considered on the merits, including (but not limited to) wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein by introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction, as in claim 2; and/or wherein the nucleic acid amplification is conducted by steps as in claims 3

and 4; and/or wherein the nucleic acid amplification is conducted at a substantially single temperature (see claim 5), in particular, where such single temperature is between 37°C and 55°C (see claim 6); and/or wherein the RNA polymerase and the second base sequence are as set forth in claim 7; and/or wherein the probe is a DNA/RNA hybrid strand (see claim 14).

By the present invention, only a single set of primers comprising a forward primer (reference character 65 in Fig. 5, for example) and a primer for introduction (reference character 52 in Fig. 5, for example) is used for analyzing one target gene. Therefore, switching temperatures during an amplification process is not necessary. Also, repeating temperature cycles essential in a polymerase chain reaction (PCR) method, as in Whitcombe, et al., is not necessary in a NASBA method, to which the present invention belongs.

By use of the primer for introduction as in the present claims, which includes the first, second and third base sequences relatively located to the 5' end of the primer, with the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, the first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, together with the probe comprising a base sequence identical or complementary to the first base sequence, a universal probe for expressed gene analysis which does not have to be designed for each use in accordance with the base sequence of the target gene is achieved. The universal probe according to the present invention can amplify and detect any type of target gene under substantially the same conditions, can detect two or more target genes derived from different samples, and analysis thereof can be simply conducted. Note, for example, the second and third

paragraphs on page 6 of Applicants' specification; see also the paragraph bridging pages 28 and 29 thereof.

Furthermore, by using at least two types of probes as in the present claims, both of the terminal bases of each module sequence being identical to each other, and each probe being constituted by rearranging the order of the module sequence having additional terminal bases, the entire sequence of each probe is different from each other, but has the same melting temperature value, and can hybridize to their complementary sequences with the same reaction properties, and therefore they can be allowed to simultaneously react in the same reaction tube, whereby accurate analysis can be made in a same reaction tube, of two or more target genes using two or more types of probes, even where the target genes are from different samples. Thus, accurate analysis can be made, when the probes are used for quantitative analysis.

Whitcombe, et al. discloses a method whereby a single TaqMan™ probe can be used for many polymerase chain reactions. The principal aim of the study reported in this article was to identify a means that the Amplification Refractory Mutation System (ARMS) could be exploited in a homogenous, high throughput and, in particular, an economical manner, and this article discloses that what was required was a way of using a single pair of allele-specific fluorescent probes for any bi-allelic polymorphism. For the fluorescent signal generation method, the authors of this article chose TaqMan™. The system used, as reported in the article, a 5'-exonuclease assay of amplicon annealed fluorogenic probes that operate in conjunction with the Amplification Refractory Mutation System, whereby relative changes in reporter fluorescent emission are monitored in real-time using an analytical thermal cycler, this system being called Three-STAR, and it is universal in

that it can either use a single probe for the detection of any one target DNA sequence or a single pair of probes for genotyping any bi-allelic polymorphism. Note the first paragraph in the left-hand column on page 918, as well as the paragraph bridging pages 918 and 919. Fig. 1 shows the scheme of the Three-STAR cycle. Note also the paragraph bridging the left- and right-hand columns on page 921, describing that the authors have devised a way to make TaqMan™ generic inasmuch as that just one fluorogenic probe can be universally applied in any PCR reaction. See also Fig. 2 on page 921, showing single-tube genotyping. Note also the legend of Fig. 1 of this article, on page 920, describing that at the start of cycle 4, the temperature switch is activated so that further priming occurs only from the Tag.

Attention is respectfully directed to the paragraph bridging pages 2 and 3 of Applicants' specification, where the teachings of Whitcombe, et al. are discussed. Note especially the problems in connection therewith. That is, in Whitcombe, et al., because two types of primer pairs are used, i.e., a primer pair of introduction and another pair hybridizing to the Tag sequence of the synthesized DNA to amplify the DNA, two different thermal cycles are necessary, which results in the unavoidable production of by-products. Moreover, when real-time detection is carried out in a single reaction vessel, reaction properties of the two types of probes cannot be precisely controlled at the same level, due to differences in Tm values of the probes.

It is respectfully submitted that Whitcombe, et al. discloses a probe non-specific to a target gene, and genotyping of two or more target genes in a single tube (see Fig. 2 and Table 1 on page 919). However, as recognized from the descriptions "homozygote" and "heterozygote" in the legend of Fig. 2, Whitcombe, et al. conducts typing of allele in a single sample. In contrast, two or more different target genes, derived from different samples, are detected in the present invention, using two or

more probes having substantially the same Tm value. As described below, two or more probes shown in Table 1 of Whitcombe, et al. have different Tm values.

Thus, Tm values were calculated using the most accurate method, base-stacking Tm calculation (http://www.promega.com/biomath/default.htm). The FAM probe and TET probe, in Fig. 2 of Whitcombe, et al., are shown below, and respectively have melting temperatures of 65°C and 72°C:

FAM probe: CTGG CATC GGTA GGGT AAGG ATCG GTAT CG, 30mer, 65°C; and

TET probe: CGGT GGAC GTGA CGGT ACGA CGAG GCGA CG, 30mer, 72°C.

When Tm values are calculated using more simple methods, Basic Tm Calculations (66°C and 71°C) and salt-adjusted Tm calculations (61°C and 66°C) show that these two probes of Whitcombe, et al., i.e., FAM probe and TET probe, have different Tm values. Moreover, from the FAM probe and TET probe as set forth in the foregoing, it is clear that the two probes used in Whitcombe, et al. do not have the structure of the probes as recited in the present claims. Thus, it is respectfully submitted that Whitcombe, et al. would have neither disclosed nor would have suggested various aspects of the present invention, including, inter alia, the probes used, including wherein the two or more probes have substantially the same melting temperature, and advantages due thereto.

The contention by the Examiner in the first full paragraph on page 5 of the Office Action mailed December 15, 2008, that Whitcombe, et al. teaches an embodiment of claim 1 wherein the melting temperatures Tm values) of the two or more types of probes are substantially the same, is respectfully traversed. As shown in the foregoing, the melting temperatures are not the same.

Even taking into account the teachings of Ovyn, et al., and Uematsu '2001, it is respectfully submitted that the combined teachings of Whitcombe, et al., Ovyn, et

al. and Uematsu '2001 as applied by the Examiner would have neither taught nor would have suggested the presently claimed subject matter, including, inter alia, the probes as recited in the present claims and as used in the presently claimed process, and target genes, and substantially same melting temperatures of the two or more types of probes.

Ovyn, et al. discloses oligonucleotides that can be used as primers to amplify a region of the 16S rRNA of *Mycoplasma pneumoniae*. See column 3, lines 39-45 of this patent. Note especially column 7, lines 5-28, of this patent, describing a method for the detection of the specified microorganism. Note also column 3, lines 39-46; column 5, lines 46-67; and column 6, lines 12-16 and 43-61.

Uematsu '2001 reports on the development of a new method that can analyze plural genes from various sources by utilizing color-selective detection coupled with size separation. The color-selective detection distinguishes the gene sources and gel electrophoresis separates the gene species. The number of fragment species that can be distinguished in an electropherogram is 10-15 by use of color detection. This article discloses that the described method is the first to use module-shuffling primers (MSPs) for comparing gene expression levels in various cells. Note the last paragraph in the right-hand column on page 1 of this article. Note also the procedure for comparing expressed genes from different sources, set forth in the second paragraph in the left-hand column on page 2 of this article. Note also the paragraph bridging the left- and right-hand columns on page 3, as well as the sole full paragraph in the right-hand column on page 3, of this article.

Even assuming, arguendo, that the teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001 were properly combinable, such combined teachings would have neither disclosed nor would have suggested a procedure as in the present

claims, including the two or more types of probes used, as recited in the present claims, with two or more target genes, derived from different specimens, being simultaneously detected in a single reaction vessel using these probes, the two or more types of probes having substantially the same melting temperatures, whereby accurate quantitative analysis of the two or more target genes, derived from different specimens, can be achieved. In this regard, it is respectfully submitted that even were the teachings of the references as applied by the Examiner properly combinable, accurate quantitative analysis of two or more different target genes, derived from different specimens, cannot be achieved from the teachings thereof. Such feature would have neither been taught nor would have been suggested by the combined teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001.

In the last paragraph on page 8 of the Office Action mailed December 15, 2008, the Examiner contends that Uematsu '2001 teaches that where each of the two or more types of probes include several module sequences of three or four bases, both of the terminal bases of each module sequence are identical to each other, and each probe is constituted by rearranging the order of the module sequences having identical terminal bases, the Examiner relying on Fig. 1 of Uematsu '2001. However, in Fig. 1 of Uematsu '2001, the primers are comprised of modules constituted of rearranged order of the module sequences. To emphasize, Fig. 1 of Uematsu '2001 shows primers, which are different from probes. Unlike probes, primers are extended by DNA polymerases. Fig. 1 of Uematsu '2001clearly states that they are "Module-shuffling Primers (MSPs)". Each of the primers shown in Fig. 1 is labeled with a fluorophore only at its 5'-terminal. It is respectfully submitted that the primers shown in Fig. 1 of Uematsu '2001 cannot work as a TaqMan probe or a molecular beacon, in which both 5'- and 3'-ends are required to

be labeled with fluorophores, or one of the ends is labeled with a fluorophore and the other is labeled with a quencher. It is respectfully submitted that it is not possible to employ the primers in Fig. 1 of Uematsu '2001, as a probe.

As can be seen in the foregoing, it is respectfully submitted that the Examiner is employing bits and pieces of the applied references, including Uematsu '2001, ignoring the teachings of these references as a whole, and using Applicants' invention as a roadmap in combining these pieces of the references. Such hindsight use of Applicants' invention is improper, and use of bits and pieces of the applied references while ignoring the teachings of these references as a whole is also improper, under the guidelines of 35 USC 103.

Accordingly, it is respectfully submitted that the teachings of Whitcombe, et al., Ovyn, et al. and Uematsu '2001, as applied by the Examiner, would have neither disclosed nor would have suggested features of the present invention as discussed previously, including the probes employed and melting temperatures thereof.

In connection with claim 14, the article by Rizzo, et al. discloses preparation of RNA/DNA chimeric molecular beacons, which contain a single-stranded RNA/DNA chimeric oligonucleotide labeled with a 5'-fluorescein as fluorophore and a 3'-DABCYL as quencher, referring to Fig. 1 on page 279 of this article. This article discloses that the fluorophore of the probe is held in proximity to the quencher by the stem-loop structure; and that when the RNA sequence of the RNA/DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested. Note the second full paragraph in the left-hand column on page 278 of this article. Note also the paragraph on pages 279 and 280; and the Conclusion set forth in the left-hand column on page 282, of this article.

Even assuming, arguendo, that the teachings of Rizzo, et al. were properly combinable with the teachings of Whitcombe, et al., Uematsu '2001, and Ovyn, et al., as applied by the Examiner, such combined teachings would have neither disclosed nor would have suggested the presently claimed method, including the two or more types of probes used, with two or more target genes, derived from different samples, being simultaneously detected in a single reaction vessel, and advantages thereof, as discussed previously, and/or such feature together with at least one of the probes being a DNA/RNA hybrid strand, as in claim 14.

It is respectfully submitted that the combined teachings of Whitcombe, et al., Uematsu '2001, and Leone 1998 (as evidenced by Leone 1997) would have neither disclosed nor would have suggested the presently claimed method.

Whitcombe, et al. and Uematsu '2001 have been previously discussed.

Leone 1998 discloses employment of molecular beacon probes in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. This article describes the coupling of RNA amplification by NASBA with amplicon detection by molecular beacon technology to produce a homogenous assay, called AmpliDet RNA. Note the first full paragraph in the left-hand column on page 2151 of this article. See also the discussions under the headings "Selection of amplification primers and probe", "Synthesis of the molecular beacons", "NASBA" and "Post-NASBA analysis", on page 2151 of this article.

Leone 1997 contains a description that the sense primers were entirely target specific, wherein the antisense primers consisted of a 3' terminal, target specific sequence and a 5' terminal T7 promoter sequence. See page 21, Section 2.2.

Even assuming, arguendo, that the teachings of Whitcombe, et al. and Uematsu '2001 were properly combinable with the teachings of the two Leone

articles, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including wherein two or more target genes, derived from different samples, are simultaneously detected in a single reaction vessel using two or more types of probes, the probes being defined as in the present claims, including, inter alia, wherein both of the terminal bases of each module sequence of the respective probes are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases, and wherein the two or more probes have substantially the same melting temperature.

The contention by the Examiner in the last paragraph on page 8 of the Office Action mailed December 15, 2008, that Uematsu '2001 teaches wherein the two or more types of "probes" have substantially the same melting temperature, is respectfully traversed. In the legend of Fig. 1 on page 2 of Uematsu '2001, this reference discloses primers, not probes, having identical melting temperatures. Such disclosure in Uematsu '2001, even in combination with the teachings of Whitcombe, et al., and Leone 1998 (as evidenced by Leone 1997), would have neither taught nor would have suggested the substantially same melting temperature of the two or more probes, and advantages due thereto.

Furthermore, it is respectfully submitted that the combined teachings of Whitcombe, et al., Uematsu '2001, the two Leone, et al. articles and Rizzo, et al., would have neither taught nor would have suggested the subject matter of claim 14.

The teachings of Whitcombe, et al. has been previously discussed, as has the teachings of each of Uematsu 2001, Leone 1998, Leone 1997 and Rizzo, et al. Even assuming, arguendo, that the teachings of these references were properly combinable, such combined teachings would have neither disclosed nor would have

suggested the presently claimed subject matter, including, inter alia, detection of two or more target genes, derived from different samples, simultaneously in a single reaction vessel using two or more types of probes, with the two or more types of probes being further defined as in the present claims, including, inter alia, wherein both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases, and with the melting temperatures of the two or more probes being substantially the same, with advantages thereof as discussed previously.

It is emphasized that according to features of the present invention, two or more different target genes derived from different samples are detected, using two or more probes having substantially the same Tm value. It is respectfully submitted that this is one of the characteristic features of the present invention. In contrast, it is respectfully submitted that Whitcombe, et al. merely described typing of an allele in a single sample. Even in light of the teachings of Leone 1998 and Leone 1997, and of Uematsu '2001, the teachings of the applied references would have neither disclosed nor would have suggested the use of two or more probes having substantially the same Tm value, or wherein two or more different target genes derived from different samples are detected, and advantages thereof.

In view of the foregoing comments and amendments, reconsideration and allowance of all claims presently being considered on the merits in the above-identified application are respectfully requested.

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Respectfully submitted,

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